

**3472-Pos Board B627****Droplet Microfluidic Technologies for High-throughput Single-Cell Gene Expression Analysis****Helmut H. Strey**, Eric Brouzes, Travis Kruse.

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In the past 5 years, droplet microfluidics has experienced tremendous growth during which mature methods for high throughput droplet manipulation have been developed. Such methods include controlled droplet generation, fusion, sorting, splitting and storage. A technological bottleneck of current droplet microfluidics is that because droplets are separated, sequential chemical reactions are more difficult to achieve. For example, in order to perform several biochemical reactions using enzymes one needs to deactivate the currently present enzymes before performing the next step. It is also much more difficult to concentrate target molecules, especially since every reaction step adds volume to the droplets.

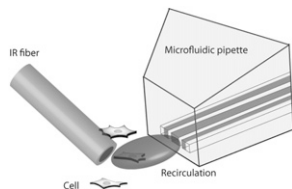
Here we developed a method to extracting and concentrating analytes within droplets in a continuous fashion by introducing functionalized magnetic microparticles into the droplets that can be manipulated by external magnetic fields and concentration is achieved by continuously splitting droplets to separate the part of the droplet that contains microparticles from the part that is devoid of particles.

We are presenting progress towards the development of a high throughput (rates up to 3000 cells/sec) microfluidics device to create cRNA libraries of single cells. This is accomplished by encapsulating single cells into picoliter droplets, extracting mRNA from the cell lysate using functionalized magnetic microparticles, and subsequently creating single cell cDNA libraries that are covalently bound to magnetic beads. Our device will outperform current techniques to study single cell gene expression by several orders of magnitude. Our approach can be used for applications in basic research (e.g. stem cell differentiation) and medicine (early cancer diagnostics and cancer treatment monitoring).

**3473-Pos Board B628****Influence of Temperature on Alkaline Phosphatase Activity in Single Cells****Shijun Xu.**

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We report a new approach for the determination of influence of temperature on enzymatic activity within a single fixed cell. We investigate enzyme activity at different temperature (22, 27, 32, 37 and 42°C) by means of directed infrared laser heating. Our method generates individual cells at selected location, through the use of a flexible microfluidic delivery device and IR fiber heating system. Using timed exposure to the pore-forming agent digitonin, we successfully controlled the extent of poration within the plasma membrane of individual NG-108 cells. Mildly permeabilized cells (~100 pores) were exposed to substrate concentrations for alkaline phosphatase and exposure to a series of temperature. We generated quantitative estimates for intracellular enzyme activity at different temperature at the single-cell level. This rapid approach offers a new methodology for characterizing influence of temperature on enzyme activity within single cells.

**3474-Pos Board B629****Layer-By-Layer Assembly of Complex Membranes****Sandro Matosevic**, Brian Paegel.

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Reconstituting complex biological macromolecular and supramolecular structure and function from simple chemical starting materials is the central goal of bottom-up synthetic biology. Phospholipid bilayer membranes, which represent a critically important class of such structures, are central to the study of signal transduction pathways, membrane transport and remodelling, yet controlled assembly of membranes remains more art than science. To address this challenge we developed layer-by-layer (LbL) membrane assembly, the sequential deposition of amphiphile monolayers on microfluidic droplet templates. We have demonstrated LbL assembly of asymmetric, double- and multi-lamellar membranes of defined phospholipid composition and structurally probed these constructs by confocal fluorescence imaging of dye-labeled lipids. Parallel measurement of protein nanopore-mediated transport revealed concerted pore insertion and leakage over multiple vesicles ( $n > 20$ ). We now demonstrate the amphiphile substrate scope of the technique through assembly and probing of vesicles composed entirely of non-ionic silicone or perfluorinated surfactant as well as minimal phospholipids mixtures that mimic

both the composition and asymmetry of prokaryotic and eukaryotic plasma membranes. Finally, we demonstrate high-resolution electron microscopy imaging-based validation of the membranes using *in situ* photopolymerization, fixing, and membrane staining. LbL assembly provides an extensible and systematic route to natural and synthetic membrane bilayers with diverse chemical configurations in a device architecture that enables structure-function studies of unparalleled reproducibility and control.

**3475-Pos Board B630****Near-Field Angular Orientation of Biological Materials****Pilgyu Kang**<sup>1</sup>, Xavier Serey<sup>2</sup>, Yih-Fan Chen<sup>3</sup>, Dakota O'Dell<sup>2</sup>, David Erickson<sup>1</sup>.

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Near-field optical techniques have enabled trapping, transport, and handling of nanoscale materials. Despite its importance, angular orientation has not been addressed by nano-optics. The angular orientation control of trapped objects provides an additional restriction on their rotational movement leaving them with one degree of freedom. This capability is well established for conventional optical tweezers, but has not been addressed in the near-field trapping literature. Near-field optical traps are of particular interest because they allow for the trapping of smaller materials than far-field optics. Additionally, in biophysical studies the orientation control of elongated objects is significant since numerous biomolecules such as DNA, viruses, and bacteria can take rod-like shapes.

Researchers have investigated ways of utilizing the polarization of the incident light to tune position of trapped particles and to continuously rotate nanoscale objects. To date no study has focused on orienting trapped objects along a fixed direction and maintaining this orientation. This is a particularly important issue to address as the size of trapped molecules keeps on decreasing. Smaller molecules sample more orientations in any given time period because of the increased rotational diffusion. This crucial effect has not been investigated yet in the recent literature on near-field trapping and should enable a better directional characterization of ever smaller biological materials.

We demonstrate the angular orientation and rotational control of biological materials with a photonic crystal resonator. Single microtubules are rotated by the optical torque resulting from the interaction with polarized electric fields. The rotational diffusion was reduced 3.7 times for an optically trapped and oriented microtubule compared to a freely diffusing one. The angular trap stiffness was determined to  $\tau = 1392 \text{ pN} \cdot \text{nm/rad}^2$  from our experiments. This study extends the functionality of near-field photonic crystal traps to enable novel biophysical studies and nanoscale physics.

**3476-Pos Board B631****Microfluidic Passive Permeability Assay using Nanoliter Droplet Interface Lipid Bilayers****Takasi Nisisako**<sup>1,2</sup>, Shiva A. Portonovo<sup>1</sup>, Jacob J. Schmidt<sup>1</sup>.

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Membrane permeability assays play an important role in assessing drug transport activities across biological membranes. However, in conventional parallel artificial membrane permeability assays (PAMPA), the membrane model used is dissimilar to biological membranes physically and chemically. Here, we describe a microfluidic passive permeability assay using droplet interface bilayers (DIBs). In a microfluidic network, nanoliter-sized donor and acceptor aqueous droplets are alternately formed in cross-flowing oil containing phospholipids. Subsequently, selective removal of oil through hydrophobic pseudo-porous sidewalls induces the contact of the lipid monolayers, creating arrayed planar DIBs between the donor and acceptor droplets. Permeation of fluorescein from the donor to the acceptor droplets was fluorometrically measured. From the measured data and a simple diffusion model we calculated the effective permeabilities of  $5.1 \times 10^{-6} \text{ cm s}^{-1}$ ,  $60.0 \times 10^{-6} \text{ cm s}^{-1}$ , and  $87.6 \times 10^{-6} \text{ cm s}^{-1}$  with donor droplets at pH values of 7.5, 6.4 and 5.4, respectively. The intrinsic permeabilities of specific monoanionic and neutral fluorescein species were obtained similarly. We also measured the permeation of caffeine in 10 min using UV microspectroscopy, obtaining a permeability of  $20.8 \times 10^{-6} \text{ cm s}^{-1}$ . Furthermore, DIBs of brain lipids were used to measure permeability coefficients of several pharmaceutical compounds. With the small solution volumes, short measurement time, and ability to measure a wide range of compounds, this device has considerable potential as a platform for high-throughput drug permeability assays.